GENETIC STUDIES ON THE LARVAL MUTANT, WHITE THORAX, IN *AEDES AEGYPTI* (Diptera : Culicidae)

THESIS SUBMITTED
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
ZOOLOGY
OF
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH

BY
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DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY, ALIGARH

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I certify that " Genetic studies on the larval mutant, white thorax, in *Aedes aegypti* (Diptera:Culicidae)", is the original work of Sibte J. Kazmi and is suitable for the award of the degree of Doctor of Philosophy of the Aligarh Muslim University, Aligarh. This work has been done by the candidate under my supervision.

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I. INTRODUCTION

Because of their medical importance, mosquitoes have been the objects of intensive research since long and a fairly vast literature has gradually accumulated regarding their systematics, distribution, bionomics, disease relationships and control. This is especially true for Aedes aegypti, the vector of urban yellow fever, dengue and haemorrhagic fever. Since the species, one of the highly evolved mosquitoes, is readily adaptable to laboratory conditions, it has been extensively used for physiological, developmental and biochemical studies. A glimpse of the vast amount of work done upto 1960 on this species is provided by Sir Richard Christopher's monumental publication, Aedes aegypti, the yellow fever mosquito, its life history, bionomics and structure (1960). How useful this small insect has been in making important contributions to the advancement of biological science has been reviewed by Craig (1965a).

Until recently, however, most of the studies on mosquitoes were confined to medical point of view. Inspite of the vast amount of work done on various aspects of the biology of mosquitoes, little consideration was given to the genetical aspects of mosquito problems. It is only during the past two decades that mosquitoes have been used extensively in genetic investigations. Mosquito genetics is, therefore, a comparatively new yet fast growing field. The first comprehensive review of the subject was made by Kitzmiller (1953) which gave the impetus for an organised research on mosquito genetics. Work on

genetic variability, insecticide resistance and cytogenetics of various species of mosquitoes has since then been undertaken by a number of laboratories. Subsequent accounts by Mattingly (1957, 1958), Rozeboom and Kitzmiller (1958), McClelland (1962a), Davidson and Mason (1963) and Kitzmiller (1963) go a long way to show how fast the literature on mosquito genetics and cytogenetics has developed during the past decade or so.

Craig and Hickey (1967), McClelland (1967) and Rai (1967) made comprehensive reviews of the genetics and cytogenetics of Aedes aegypti in the book, Genetics of insect vectors of disease, published for the World Health Organisation. This now serves as a distinct landmark in the genetic research of Aedes aegypti. These studies seem to have reached a point where mosquitoes are providing tools for solution of basic biological problems and genetics is providing tools for a better understanding and probably solution of many mosquito problems.

Due to certain advantages over other species of mosquitoes, Aedes aegypti has been able to get much more attention in the laboratories of the world. Being a plastic species, it can be reared at varying temperatures. The breeding behaviour permits large numbers of stocks to be reared and maintained in the laboratory without much difficulty. The eggs can be readily stored and remain viable on dry papers for six to eight months. These can easily be transported to any laboratory in ordinary envelopes. The life cycle is comparatively short and is completed in about two weeks.

Like other mosquitoes Aedes aegypti also possesses only three pairs of chromosomes which, being sufficiently large, can be easily manipulated. These advantages make Aedes aegypti a particularly good species for genetic research and, like Drosophila, it has all the potentialities of providing valuable information to our basic knowledge of genetic mechanisms.

At present a large number of workers are engaged in different laboratories of the world on various aspects of Aedes genetics. Research is in progress on the genetics of insecticide resistance, vector ability, population genetics, cytoplasmic inheritance, biochemical and physiological genetics and the genetic effects of radiation and chemosterilants. Efforts are being made to develop a formal genetics of the species and take it to the level of Drosophila genetics, perhaps even farther. Even complicated areas like the genetics of behaviour have not remained untouched and work is proceeding with considerable speed (Mattingly, 1967; Schoenig, 1967, 1968, 1969).

Extensive but not exhaustive work of the pioneers of Aedes genetics, Craig and his coworkers, has belied Christopher's remark (1960) that Aedes aegypti is not a variable species. Craig and VandeHey (1962) reported a large number of variants in Aedes aegypti and according to Craig and Hickey (1967), "The morphological variability in various geographical populations of Aedes aegypti is so extensive that designation of 'normal' or wild type phenotype becomes very difficult." Approximately 87 morphological, structural and colour

mutants have been isolated and linkage maps have been constructed (W.H.O. Scientific Group, 1964). Leaving monozygotic twins, individuals of every species vary in large number of respects. All such variations between individuals are, however, not necessarily hereditary and only a portion of the variability may be genetic. Genetic variability confers plasticity which may be beneficial to both, species and the individual. According to Craig (1964), "The more kinds of genes present in the gene pool, the greater is the possibility for the adaptation to diverse environment".

The majority and most of the mutants reported in Aedes aegypti concern characteristics of adult mosquitoes. Larval mutants are rather rare and only two, one affecting colour (Craig and Gillham, 1959) and the other affecting structure of the larvae (Leahy, 1967), have been reported so far. It is, however, interesting to note that inspite of the rarity of larval mutants in Aedes aegypti, the first mutant discovered was a gene affecting larval colour. It was first reported by Craig and Gillham (1959). They successfully isolated four distinct types of colour variants amongst the larvae. The wild type or predominant class is dark grey in colour. The most common variant is a yellowish and pale form. The less common form, brownish, has been reported only in strains from Southeast Asia. The fourth type, rather rare in most strains, consists of melanotic larvae which are dark brown to deep black. Craig and Gillham further demonstrated that the yellow phenotype is controlled by an autosomal recessive gene and designated it on linkage group II. The four forms have been described

as members of a multiple allelic series, y^+ , y , y^{br} and y^m , respectively.

The cuticle of mosquito larvae being transparent, the colour of the larvae is determined by the nature of the parietal fat body. The uric acid granules stored in the cells of the larval fat body and described by Wigglesworth (1942) as an excretory mechanism, were suggested by Craig and Gillham (1959) to be responsible for the colour variation of the larvae. The suggestion has been confirmed by Adhami (1964) in his genetic studies on the yellow larva.

Pigmentation in mosquito larvae has earlier been studied by a number of workers. Most of these studies had been mainly confined to Anopheles quadrimaculatus and Culex pipiens complex. Coggeshall (1941) suggested polygenic inheritance for white striping in the larvae and pupae of Anopheles quadrimaculatus. Yellow, pink, green, gray-green and melanotic larvae have been described in the Culex pipiens complex (Huff, 1929 ; De Boissezon, 1930 ; Ghelelovitch, 1950 ; Kitzmiller, 1953 ; Laven and Chen, 1955 ; Spielman, 1957). Monofactorial, autosomal inheritance was reported for all these colour variants. Craig and Gillham (1959) reviewed this work and compared them with their yellow larval mutant in Aedes aegypti. The colour variation in Aedes aegypti is controlled by the presence, relative number or complete absence of discrete granules in the cells of the fat body. The same is not true in case of Culex and Anopheles. The different forms described by Craig and Gillham (1959) were found to be allelic while none of the various factors responsible for larval

pigmentation in Culex and Anopheles were related to each other.

A new addition to the larval colour variants in Aedes aegypti is being introduced in the present work. These larvae have a pale or white thorax while the rest of the body has normal pigmentation and, being dark gray in colour, looks more or less like the wild type. The mutant was first discovered by the author in the ROCK strain during the course of routine breeding programme of different strains of Aedes aegypti. It was isolated and maintained in the laboratory by inbreeding. Subsequently some other strains also showed the presence of the mutant larvae. It has been designated as the white thorax larval mutant (Figure 1). It differs from the yellow larva rather markedly. In these larvae only the thorax is devoid of colour and looks pale or white while in the yellow mutant the whole larval body is albinistic. It is also very different from the bronze mutant described by Bhalla and Craig (1964). Even though bronze is an adult mutant, the colour variation is also expressed in the larvae where the whole of the larval body, including the head capsule, air tube and annal saddle, turn to a pale tan or bronze colour.

Preliminary experiments showed white thorax larval character to be an autosomal, recessive hereditary trait. The symbol wt, w for white and t for thorax, has been assigned to the gene controlling this trait. The wild type for this character has consequently been designated as wt^+.

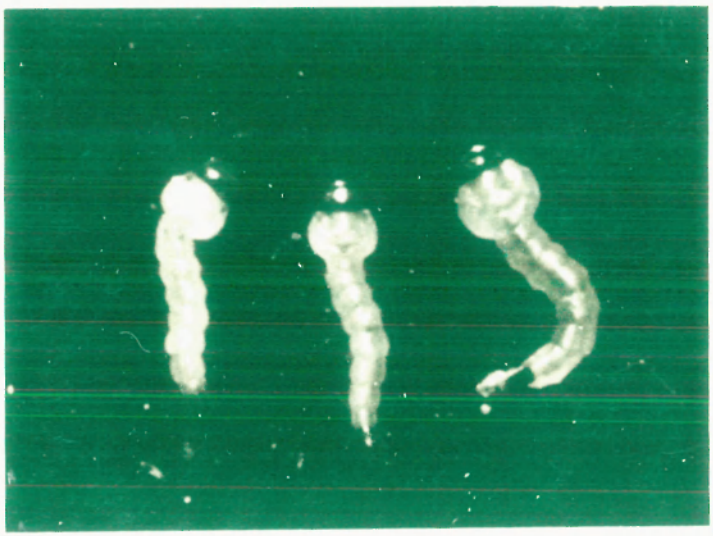
The present investigation can roughly be divided into three

Figue 1. Colour forms of larvae of Aedes aegypti (ROCK strain).

A. The mutant white thorax larva;

B. Yellow larva and

C. Wild type larva.



A B C

parts: First, a study of the nature and immediate cause of the white thorax phenotype was made and the frequency of the mutant was studied in four different strains of Aedes aegypti. Second, the mechanism of inheritance and the linkage group of the wt gene were worked out. Third, factors which might favour one of the two phenotypes were investigated.

The work on the mechanism of inheritance is an addition to our existing knowledge for the vast amount of information which would be needed to complete the mapping process. It may thus be expected to contribute to the development of a formal genetics of the species. Biochemical assays and comparison between the present mutant and the yellow larvae may also provide an insight into the physiological processes where two different genes may produce similar effects on different parts of the body. It is also hoped that the results of the present work would make some addition to our knowledge of the importance of various selective forces which might affect the frequencies of certain genes in field and laboratory populations.

II. MATERIALS AND METHODS

Strains: From the various strains of Aedes aegypti obtained from the Vector Biology Laboratory, University of Notre Dame, Indiana, U.S.A., and maintained at the Mosquito Genetics Laboratory, Aligarh Muslim University, four strains ROCK, NIH, KUALA and NEW MICK were used for the present work. All these have been reared in the laboratory for quite some time (Table 1). ROCK was selected because it was this strain in which the mutant was first isolated. Since Adhami (1964) indicated differential mutation in different strains of Aedes aegypti, NIH, KUALA and NEW MICK were used for comparative work on this larval mutant.

Description of mutant: While a colony of ROCK strain was being maintained, a very clear new larval mutant was observed. In this mutant the thorax of the larvae appears white which is clearly different from the thorax of wild type which is dark (Figure 1). It also differs from the mutant yellow larva reported by Craig and Gillham (1959) which Adhami (1964) showed to be due to the difference in deposition of the granules of uric acid in the cells of fat body. The mutant larva, reported here, has white thorax while other parts of the body are of the usual wild type. Almost in all the wild type forms the thorax is entirely of the same colour as the body. In this sense it significantly deviates from the wild type. The present mutant is also quite different from the bronze mutant (Bhalla and Craig, 1964) whose larvae are completely pale and phenotypically

Table 1: Strains of Aedes aegypti used for studies on white thorax larva

Name	Source	Strain history	% of yellow larva	% of white thorax larva
NIH	Prof. G.B. Craig, Vector Biology Laboratory, Univ. of Notre Dame, Indiana, U.S.A.	Laboratory rearing at least 22 years. Maintained at the Mosquito Genetics Laboratory Aligarh Muslim University, since 1967.	14.0 *	16.0 o
ROCK	Prof. G.B. Craig, Vector Biology Laboratory, Univ. of Notre Dame, Indiana, U.S.A.	Laboratory rearing at least 26 years. Maintained at the Mosquito Genetics Laboratory Aligarh Muslim University, since 1967.	25.0 **	43.0 o
KUALA	Prof. G.B. Craig, Vector Biology Laboratory, Univ. of Notre Dame, Indiana, U.S.A.	Laboratory rearing at least 14 years. Maintained at the Mosquito Genetics Laboratory Aligarh Muslim University, since 1967.	7.0 ***	4.0 o
NEW MICK	Prof. G.B. Craig, Vector Biology Laboratory, Univ. of Notre Dame, Indiana, U.S.A.	Laboratory rearing at least 11 years. Maintained at the Mosquito Genetics Laboratory Aligarh Muslim University, since 1967.	14.0 ***	10.0 o

* Craig and Gillham, 1959

** Craig, VandeHey and Hickey, 1962

*** Strain list of 1967 issued from the Mosquito Genetics Project, Department of Biology, University of Notre Dame Notre Dame, Indiana, U.S.A.

o Present work, count based on at least 2,000 individuals.

resemble the yellow larvae.

A very characteristic feature of this mutant is that it does not differ from the wild type larvae in the early stages of development. It is only when these larvae reach third instar that the differentiation becomes conspicuous. At this stage the thorax of these larvae becomes white leaving the rest of the body dark brown or characteristically wild type. Its detection, therefore, is very easy at this stage.

All these larvae were isolated from the stock and reared in separate pans. A separate colony was established for them by mass breeding and some single-pair crosses were made to detect the mechanism of inheritance, if any. Preliminary tests showed that the character was hereditary and of a monofactorial recessive nature. The mode of inheritance is described in detail in Chapter III.

To ensure homozygosity wild type lines were tested by backcross method. Pure lines thus obtained were massed separately for maintaining stock cultures of each genotype. Further experiments were done with the stock cultures of $\underline{+}\underline{+}$ and $\underline{wt}\ \underline{wt}$ thus produced for this strain ($\underline{+}$ symbol, instead of $\underline{wt}^{+}$, is being used here to represent the wild type allele). Studies on the heterozygotes were done with eggs obtained from mass crosses between pure lines for each colour. These crosses were made in each direction and the progeny from reciprocal crosses were pooled for testing. This is legitimate because of the indication that an autosomal gene is involved here. According to the data discussed later, there was no evidence that the character is sex-linked

or sex-influenced.

Rearing Methods: Deoxygenated water was used for hatching. The eggs were immersed over-night to ensure complete hatching of viable eggs. The genetic factor of late hatching, described by Gillet (1959), which was not found by Adhami (1964), was detected in this mutant from all the strains used. Larvae were reared in enamel pans of 12" diameter. They were fed on powdered yeast tablets of Alembic Chemicals throughout the experiments. The powder quickly settles down at the bottom making the feeding easy for the larvae, Christophers (1960). Bacot (1916) and Atkin and Bacot (1917) found that yeast is an essential requirement for the larvae of mosquitoes. Adults were fed on sugar pads and anesthetized albino rats twice a week. An injection of Sodium Pentabarbitol (Nembutol) of Abbot Laboratory was used as anesthesia for these albino rats. The egg papers were retained for 72 hours before hatching for conditioning and embryonation under optimal conditions. Where mentioned otherwise, the term optimal condition denotes 27° C $\pm$ 1° C and 80% $\pm$ 10% relative humidity. To avoid overcrowding not more than 50 to 70 pupae were kept in an enamel mug and 300 to 400 larvae in an enamel pan (Adhami, 1964). This gave approximately 1 square centimeter of surface area for each pupa in the mug while the pan provided approximately 1.5 square centimeter of surface area for each larva. Extra mugs were introduced in population cages to provide more area for emergence and oviposition.

The number of yeast tablets given at a time depended on the

number of larvae in the pan. Generally one tablet was found to suffice for 300 larvae on the first day. In order to avoid scum formation, the water of the pan was changed whenever required.

For the majority and most of the experiments the following feeding schedule was found to be satisfactory.

Day 0----------No food

Day 1----------One tablet

Day 3----------One tablet

Day 4----------One tablet

Day 6----------One tablet

The rearing and feeding schedule and all the precautions were the same as that of Craig and VandeHey (1962) and Adhami (1964). All the experiments were done in an environmental control room with constant conditions of 27° C ± 1° C and 80% ± 10% relative humidity. The rearing procedure was kept constant throughout this work.

Counting of phenotypes: The counting of phenotypes becomes comparatively easier due to marked differentiation of the colour of the thorax. It can be done without the help of a microscope or binocular in later stages of the larvae i.e. in the third and fourth instars where colour differentiation becomes very conspicuous.

Rearing methods for population study: Two separate cages of + + and wt wt adults were maintained in order to study the behaviour of the wt gene in populations. The cages were of two sizes, one measuring 15" X 15" X 15" to accomodate more than 1,000 mosquitoes, while the other measuring 12" X 12" X 12" for less than 1,000 mosquitoes

(Figure 2). These wooden cages had wire mesh fixed to three sides while the front side had a plain glass in order to observe the mosquitoes. An arrangement of sleeve was also there to put the pupae inside the cage and to take out the egg papers. An enamel mug of $3\frac{1}{2}$" depth and $3\frac{1}{2}$" in diameter was pupal emergence. The same was lined with paper towelling for oviposition. After using the mugs once for emergence, these were always sterilized to avoid contamination before the next use. The same procedure was followed for the larval rearing pans. Adults were studied under the binocular after anesthetizing them with ether.

Figure 2. The apparatus and cages used for breeding.

A. Population cage for more than 1,000 mosquitoes.

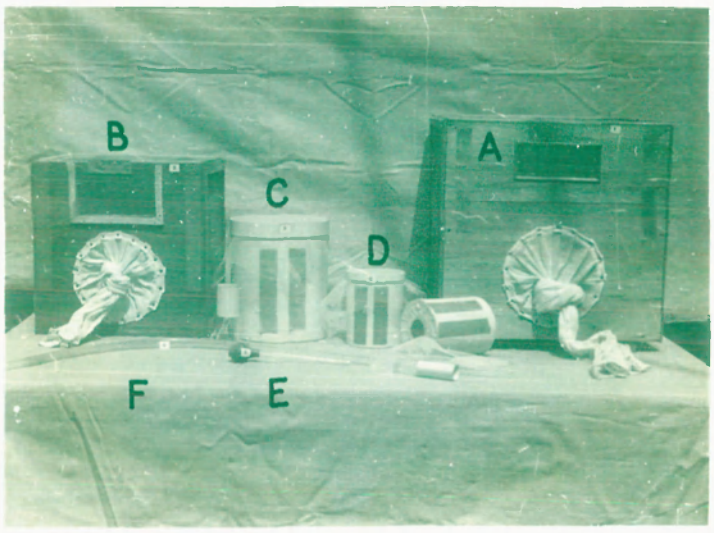
B. Population cage upto 1,000 mosquitoes.

C. Gallon cage for the progeny of single-pair crosses.

D. Single-pair cage and its accessories.

E. The dropper for picking the required larvae and pupae.

F. Aspirator for picking the adult mosquitoes.


B
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III. NATURE OF PHENOTYPIC VARIATION

A good amount of literature on the colour variation in mosquito larvae has been contributed by a number of workers. Imms (1908) reported the presence of olive granules in the fat cells in Anopheles maculipenis. De Biossezon (1930) described them to be of purine nature in Culex pipiens. Wigglesworth (1942) reported the presence of uric acid granules in the larval fat body of mosquitoes as an excretory mechanism. Later, Craig and Gillham (1959) made it the cause of colour variation in mosquito larvae of Aedes aegypti ranging from white to dark grey. Adhami (1964) while describing the nature of yellow larva in the NIH strain of A. aegypti confirmed the hypothesis that these uric acid granules are the immediate cause of colour variation in the larvae of Aedes aegypti.

To study the cause of this colour variation in white thorax larvae same experiments were performed as to Adhami (1964).

One hundred larvae of first instar of each genotype + + and wt wt from ROCK strain of Aedes aegypti were starved for 20 days. The larvae were kept in distilled water which was changed every alternate day. In the beginning first and second instars of both the genotypes appeared yellow. The 3rd & 4th instar larvae were examined under a microscope every day and the changes taking place in them are reported in Table 2.

Upto day 2 the third instar larvae of both the genotypes did not show the deposition of any uric acid granules. From day 3 to 8 the

Table 2: Progressive changes in the amount of uric acid granules during starvation (ROCK strain).

Larval instars	Time in days	Phenotypes*	
		± ±	wt wt
Late 2nd	1-2	-	-
	3-8	+	-
Early 3rd	9-12	+ + +	+
4th	13-14	+ + + +	+ + +
	15	+ + + + +	+ + +
	16	+ + + + + +	-
	17	+ + +	-
	18	+	-
	19	-	-
	20	-	-

*-Signs denote the absence of granules while the + signs denote the presence and relative amount of uric acid granules in fat body cells.

wild type larvae showed minute granules by the side of the fat body. The amount of the granules remained more or less constant from day 3-8. There were no granules in the mutant larvae even at this stage. From day 9 the amount of granules started increasing in the wild type larvae. The increase continued upto day 16 after which there was again a sudden decrease in the number of granules which had completely vanished on the 19th and 20th days. The mutant larvae also showed the presence of a little amount of these granules from day 9-15 but the distribution of the granules was not uniform. The granules were present only in the cells of the abdomen while the thorax was completely devoid of them. This is in sharp distinction to the distribution of these granules in the wild type as well as in the yellow larvae (Adhami, 1964).

Most of the larvae of both the genotypes had lost their vitality after the sixteenth day as they appeared to be very sluggish. Mortality also increased tremendously and only 15-20 of the 100 larvae of each genotype survived upto the 20th day of starvation when there were no granules at all in any region of the body in both the genotypes.

The colour of the larvae was closely linked with these granules. The larvae became darker with the increased accumulation of these granules. Since there was no accumulation of the granules in the thorax of the mutant larvae it did not appear to be dark at any time of the whole experiment. The contrast was remarkable from day 9-15 when the abdomen of the mutant larvae assumed a somewhat grey colour but the thorax remained white or colourless. Larvae of both the genotypes reverted to lighter or pale during the last stages of

starvation when the granules disappeared.

Unstarved, fourth instar larvae of both the genotypes were fixed for six hours in aqueous Bouin's solution. They were then dissected in 90% alcohol and were mounted in Euparal. Small sheaves of needle-like crystals, described by Wigglesworth (1942) as uric acid were seen in the thorax as well as in the abdomen of wild type larvae (Figure 3a). These crystals were found in reduced numbers in the abdomen of the mutant larvae but were absent in the thoracic region (Figure 3b). Their presence in the abdomen and complete absence in the thoracic region of the unstarved mutant larvae seems to be a strong evidence for the part played by them in colour variation.

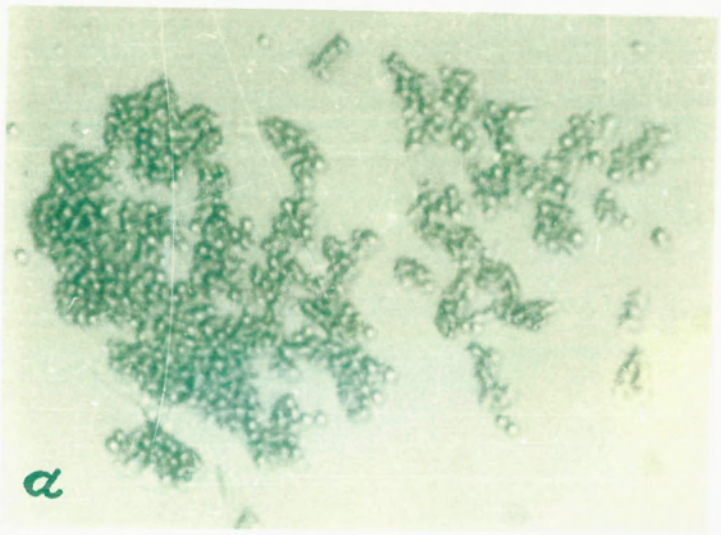
The chemical nature of these granules was also studied in the 4th instar stage of both the phenotypes by staining them with carmine and methylene blue using Schultz-Schmitz stain for sodium urate (Gurr, 1956). The deep greenish-blue dots characteristic of uric acid, were found at the site of dark granules in the wild type larvae (Figure 4a). The mutant larvae also showed such dots in the abdominal segments but there was no stain in the thoracic region of these larvae (Figure 4b). The fat body in the thorax of these larvae appeared to be almost transparent.

From the above mentioned tests and experiments it is evident that crystals of uric acid are playing a key role in the determination of larval colour as reported by Adhami (1964) in the mutant yellow larva. There the whole body appeared to be pale or yellow due to the absence of the crystals of uric acid from all the regions. Here only thorax is affected, as it accumulates very little or no granules at all.

Figure 3. Fat body cells of fourth instar larvae fixed in aqueous Bouin's solution (ROCK strain).

a. Wild type larvae showing sheaves of uric acid crystals.

b. Translucent cells of white thorax larva, lacking crystals of uric acid.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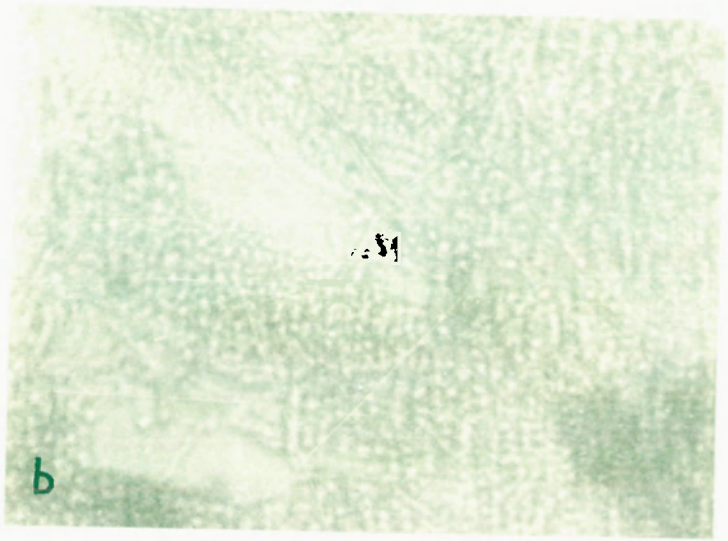
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Figure 4. Fat body cells of fourth instar larvae stained with carmine and methylene-blue (ROCK strain).

a. Wild type cells showing stained (deep greenish blue) dots of uric acid.

b. Translucent cells of white thorax larva lacking dots.

IV. MECHANISM OF INHERITANCE

To determine the factor responsible for white thorax larva and to compare it with yellow larva, separate strains of white thorax, yellow and wild type larvae (in both regards) were isolated and inbred for five to eight generations. These strains bred true for their respective larval colours.

Five single-pair crosses were made with inbred white thorax females and wild type males. Five reciprocal crosses were also made. The F1 individuals, in every case, were all dark or having wild type larval pigmentation (Table 3). These results were duplicated several times.

Two or three brother-sister crosses were made from F1 of each of the original crosses. The phenotypic ratio of the F2 progeny so obtained are shown in Table 3 along with the X^2 values calculated from an expected ratio of wild type to white thorax of 3:1. Crosses that failed to fit the expected ratio of 3:1 at the 5 per cent level of confidence are indicated with an x. As would be clear from the table, only two out of the 23 crosses failed to fit the hypothesis. Even in these two cases the departure was not extreme.

The F2 ratios of 3:1 in both type of crosses lead to two satisfactory conclusions. Firstly, the gene wt responsible for giving rise to white thorax larva is a recessive one. It is dominated by its allele wt^+. Secondly, sex of the parent bearing this character does not in any way modifies its distribution in

Table 3: Segregation of larval colour in the F2 from single-pair crosses between inbred lines of wild type and white thorax larvae (ROCK strain).

Parental cross	F1 Phenotype		F2 Phenotype		X^2	Fails to fit 3:1 ratio at 5% level
	wild type	white thorax	wild type	white thorax		
white thorax ♀♀ x wild type ♂♂	80	0	23	7	0.044	
			84	25	0.197	
	65	0	46	15	0.000	
			91	29	0.044	
			65	20	0.064	
	71	0	72	20	0.521	
			90	18	4.000	x
			30	8	0.316	
	81	0	71	29	0.853	
			51	15	0.181	
	35	0	50	19	0.312	
			29	10	0.000	
			68	21	0.060	
wild type ♀♀ x white thorax ♂♂	90	0	96	19	4.611	x
			49	15	0.083	
	81	0	79	21	0.853	
			89	30	0.000	
	107	0	42	13	0.095	
			91	36	0.612	
			95	30	0.042	
	98	0	61	29	2.503	
			52	16	0.078	
	69	0	64	21	0.000	
			59	20	0.000	
			78	25	0.051	

the offspring. Had it been sex-linked or sex-influenced, there would have been different results in reciprocity. Since similar results were obtained in both types of crosses the possibility of the gene wt being on linkage group I is ruled out. The results confirm the hypothesis that wild type and white thorax larval colouration in Aedes aegypti are caused by a single pair of autosomal alleles with wild type being dominant to white thorax.

In order to test the F1 progeny, some females and males of the F1 were backcrossed to inbred white thorax males and females. The results are shown in Table 4 together with X^2 values derived from an expected ratio of 1:1 for the backcross progeny. None of the nine crosses failed to fit the expected (1:1) ratio at the 5 per cent level of confidence. The results suggest that the production of phenocopies is not at play in this larval mutant as has been reported by Craig and Gillham (1959) for the heterozygotes of the yellow larva.

In order to determine the linkage group of the wt gene certain crosses with markers had to be made. The gene y for yellow larva, designated on linkage group II by Craig and Gillham (1959), has proved to be a good marker for studies on linkage relationships of various genes. Khan and Brown (1961) and Brown and Abidi (1962) used the gene y to determine the mode of inheritance of Dieldrin-resistance and DDT-resistance in Aedes aegypti. The same gene y, responsible for yellow larva, was used in the breeding experiments to find out if the wt gene

Table 4: Segregation of larval colour in the backcross from single-pair crosses between inbred lines of wild type and white thorax larvae (ROCK strain).

Parental type	Backcross	Backcross progeny: wild type	Backcross progeny: white thorax	X^2	Fails to fit 1:1 ratio at 5% level
white thorax ♀♀ X wild type ♂♂	F_1 ♀♀ X white thorax ♂♂	47	37	1.190	
		51	58	0.450	
	white thorax ♀♀ X F_1 ♂♂	22	18	0.400	
		44	32	1.894	
wild type ♀♀ X white thorax ♂♂	F_1 ♀♀ X white thorax ♂♂	40	52	1.564	
		60	44	2.460	
	white thorax ♀♀ X F_1 ♂♂	31	26	0.438	
		22	28	0.720	
		62	48	1.782	

was also located on chromosome II.

It was observed during the course of breeding experiments that in the presence of yellow larval phenotype, the white larval thorax could not be identified in the same individual. Since both characters affect the larval body colour, a double mutant always looked like a pure yellow larva, as if masking the white thorax phenotype. There was thus no way to distinguish between the double mutants and the yellow larvae. Since a double mutant showing both the larval characters could not be obtained, it was not possible to make a test cross with the double homozygous recessive characters.

Five single-pair crosses were made with inbred white thorax females and yellow males. The same number of reciprocal crosses were also made. As expected, the F1 larvae were all dark or wild type in the progeny of all the crosses (Table 5). From the F1 progeny of each original cross, two or three brother-sister crosses were made to get the F2. The phenotypic ratios of the F2 progeny are shown in Table 5. Each one of the 21 crosses gave a phenotypic ratio of 2:1:1 instead of a 3:1 ratio. The results, therefore, are indicative that the two pairs of alleles are not segregating independently but are linked together. Even though the ratio of yellow to white thorax phenotypes is very close to 1:1, it is striking to note that except for two crosses, the number of yellow was slightly higher than those of the white thorax larvae in every case in the F2 progeny. The data of Table 5

Table 5: Segregation of larval colour in the F2 from single-pair crosses between inbred lines of yellow larvae and white thorax larvae (ROCK strain).

Parental cross	F1 Phenotypes			F2 Phenotypes			
	wild type	yellow	white thorax	wild type	yellow	white thorax	yellow & white thorax
white thorax ♀♀ X yellow ♂♂	97	0	0	40	23	17	0
				44	25	16	0
	79	0	0	39	20	17	0
				28	16	14	0
	98	0	0	52	24	24	0
				46	26	22	0
	78	0	0	39	21	19	0
				34	18	12	0
	88	0	0	44	19	21	0
				50	24	21	0
				43	25	20	0
yellow ♀♀ X white thorax ♂♂	92	0	0	43	21	20	0
				53	28	22	0
	100	0	0	36	21	19	0
				54	30	26	0
	86	0	0	33	15	16	0
				40	25	22	0
	70	0	0	36	17	16	0
				30	18	16	0
	96	0	0	53	26	21	0
				45	25	24	0

has been summarized in Table 6 where this difference in the number of yellow and white thorax larvae becomes more conspicuous. If the two genes are located on the same linkage group, they must also show new combinations in the progeny which would be produced as a result of crossing over between the two loci. The new combinations would inevitably result in the formation of individuals having both the mutant characters, yellow and white thorax larvae. But since such a double mutant can not be phenotypically differentiated from the yellow larvae, all such cases of double mutants formed as a result of crossing over, must have been scored with the yellow phenotype. This would explain satisfactorily the complete absence of double mutants and slight increase in the number of yellow phenotypes in the F2 progeny (Table 5 and Table 6).

Since a cross between the white and yellow larval genotypes always produced only wild type phenotypes in F1 and not any intermediates between the two larval characters, the possibility of the two characters being alleles can easily be ruled out.

We would be justified to conclude, from the results given above, that the gene for white thorax larval colour (wt) is linked with the gene y (for yellow larva) and therefore lies on linkage group II.

Since more markers were not available the distance relationship of the wt gene with other loci could not be ascertained.

Table 6: Summarized data of Table 5, showing segregation of larval colour from single-pair crosses between inbred lines of yellow larvae and white thorax larvae (ROCK strain).

Parental cross	F1 Phenotypes*				F2 Phenotypes**				
	Total no.	wild type	yellow	white thorax	Total no.	wild type	yellow	white thorax	yellow white thorax
white thorax ♀♀ X yellow ♂♂	440	440	0	0	903	459	241	203	0
yellow ♀♀ X white thorax ♂♂	444	444	0	0	851	423	226	202	0

*Based on the total progeny of 5 single-pair crosses.

**Based on the total progeny of 12 single-pair crosses.

Back Mutation

Since the white thorax larval character is due to a mutation from $\underline{wt}^+$ to $\underline{wt}$, evidence of reverse mutation from $\underline{wt}$ to $\underline{wt}^+$ was sought. Lines homozygous for white thorax were inbred for five generations by single pair matings of the two strains ROCK and NIH. These were then expanded by mass crosses with one hundred adults of each sex in a population cage. Separate cages and larval pans were maintained for the ROCK and NIH strains and the experiment was conducted under optimal conditions of feeding and environment.

Larval phenotype was examined for seven filial generations of the mass crosses (Table 7). Since white thorax larva is a recessive trait, the appearance of a single wild type individual would indicate a mutation from $\underline{wt}$ to $\underline{wt}^+$. A total of 21,632 larvae were examined from the two strains. No wild type individuals were found. This represents no mutation from $\underline{wt}$ to $\underline{wt}^+$ among 43,264 gametes tested. Obviously a reverse mutation seems to be rare.

Table 7: Frequency of back mutation (wt to wt^+) as observed in the progeny of mass crosses in sequential generations.

Generations	No. of larvae examined in strains*			
	ROCK		NTH	
	Total	±	Total	±
F_1	1,305	0	1,150	0
F_2	1,953	0	2,515	0
F_3	869	0	3,001	0
F_4	1,812	0	841	0
F_5	1,918	0	751	0
F_6	1,105	0	1,101	0
F_7	2,003	0	1,308	0

*± = number with wild type phenotype.

V. FREQUENCY OF THE GENE wt IN POPULATIONS

Craig, VandeHey and Hickey (1961) showed that the frequency of the gene for yellow larva varied in different strains of A. aegypti reared under laboratory conditions. Adhami (1962) reported a change in the gene frequency of yellow larva in continuous populations of the same strain over a period of time. Adhami (1964) made a comparative study of the amount of change in various strains reared in a single laboratory and found that the rate of change varied in different populations.

In order to determine the frequency of the gene for white thorax in different populations the strains ROCK, KUALA, NIH and NEW MICK were used. The eggs of these strains were originally obtained from the Vector Biology Laboratory, University of Notre Dame, U.S.A. and have been maintained in this laboratory by mass breeding since 1967 (Table 1). The eggs were hatched and reared under optimal conditions. Progeny counts of the larvae for yellow and white thorax were made at the third and fourth instar stages. Gene frequencies for both the larval mutants were calculated from the Hardy-Weinberg formula. Table 8 thus gives a comparative account of the present frequencies of the two genes in the four strains maintained in this laboratory.

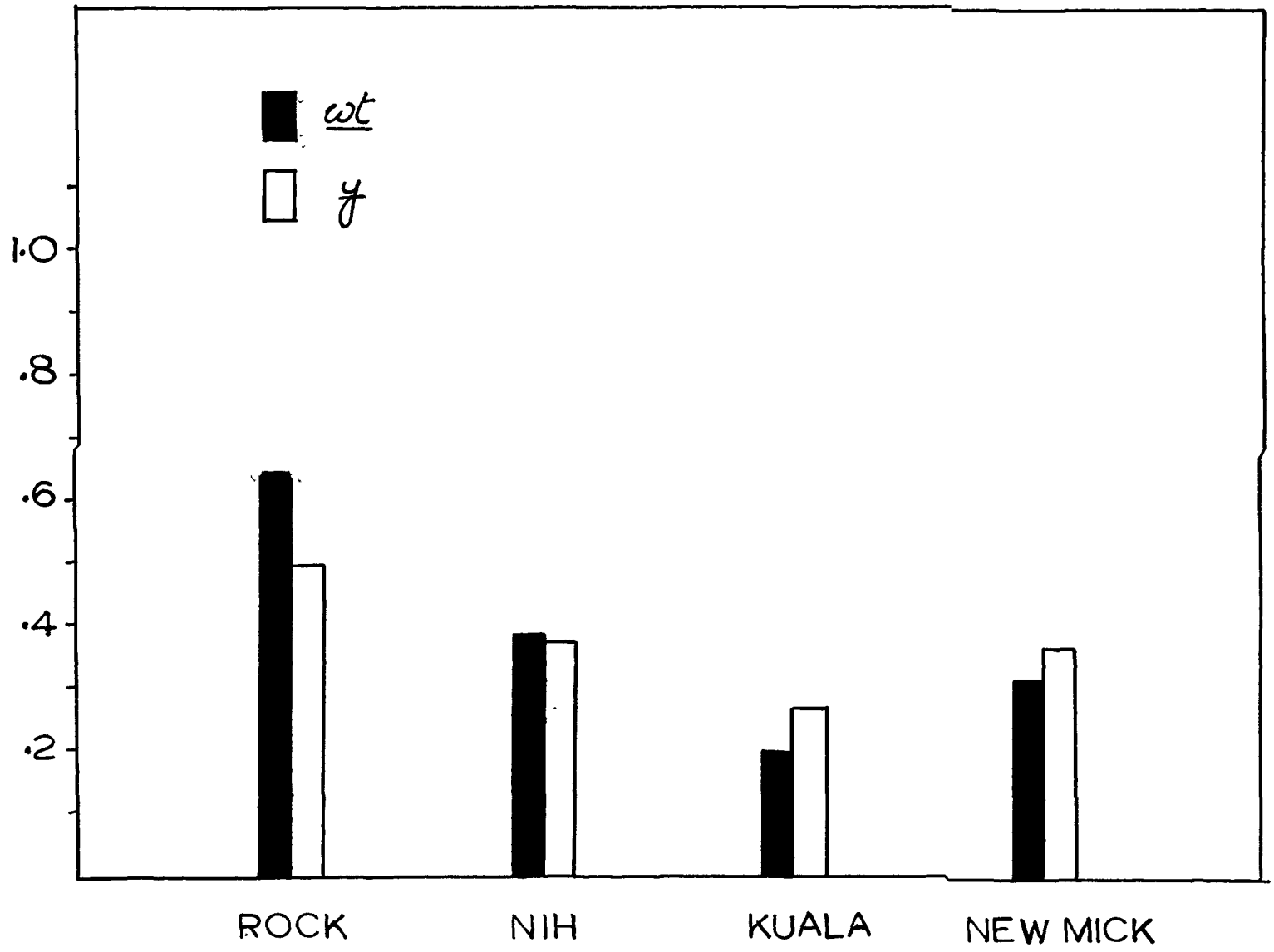
As is evident from the data (Table 8, Figure 5) the four strains differ markedly in the frequency of wt gene. The highest frequency (0.65) was found in the ROCK strain and the lowest (0.20)

Table 8: Frequency of the genes for white thorax (wt) and yellow larva (y) in laboratory colonies of Aedes aegypti.

STRAIN	Proportion of the two larval mutants*			
	% white thorax phenotypes	% yellow phenotypes	Frequency of the gene in population	
			wt	y
ROCK	42.3	25.0	0.65	0.50
NIH	15.2	14.2	0.39	0.38
KUALA	4.0	7.2	0.20	0.27
NEW MICK	10.0	13.9	0.32	0.37

*Each count represents more than 2,000 larvae.

Figure 5. Frequency of wt and y genes in laboratory colonies of Aedes aegypti.


wt
y
1.0
.8
.6
.4
.2
ROCK
NIH
KUALA
NEW MICK

in the KUALA. NIH and NEW MICK had intermediate values 0.39 and 0.32 respectively. The frequency of the gene y also varied in the four strains but the difference did not seem to be so marked as in the case of the gene for white thorax.

VI. COMPETITIVE ABILITY OF THE GENOTYPES

Craig and Gillham (1959) and Adhami (1962) reported that the gene (y) for the yellow larval mutant was disadvantageous under some conditions. Adhami (1964), however, showed that the gene also bestowed certain advantages on the mutant and the factors favouring yellow included better survival of the larvae when food was insufficient, adult longevity and mating efficiency. Since the present work also deals with a larval mutant, efforts were made for a comparative study of the selective advantage or competitive ability of the genotypes at different stages of the life cycles and under different conditions.

A. Oviposition

Gillet (1955c, 1956a) while investigating the effect of mating on oviposition in Aedes aegypti, reported a marked difference in oviposition behaviour between the two strains LAGOS and NEWALA. The differences were found to have a genetic basis.

In order to study the differences, if any, in the oviposition behaviour sixty single pair crosses were made for each genotype (+ + and wt wt) from the ROCK strain. The adults used were of uniform age (72 ± 12 hours) and were kept under optimal conditions. Blood meal was provided from anesthetized albino rats on the 5th day of emergence (Craig and VandeHey, 1962) and glucose pads were made available at all times.

In order to determine the time taken for oviposition by the females of the two genotypes after the blood meal, egg papers were

removed from the cages every morning and evening and a 12-hourly record of the ovipositing females was maintained. In case of mortality of either sex the cage was discarded and was not included in the count.

As the data (Table 9 and Figure 6) indicate there is a marked difference in the oviposition time of the females of the two genotypes. The mutant females took much longer time (120.9 ± 1.26 hours) in laying eggs than the wild type females (99.9 ± 0.9 hours). The results are statistically significant and indicate that the females of the mutant have a tendency for late oviposition as compared to the females of the wild genotype.

Effect of repeated blood meals on survival & oviposition:

Lavoipierre (1961) noted that mortality of adult female mosquitoes was related to the development of ovary. Clements (1963) found that in Aedes aegypti mortality of the females increased with successive egg layings due to repeated gonotrophic cycles. An experiment was designed to study the effect of repeated blood meals on the survival and oviposition of females of the two genotypes (+ + and wt wt).

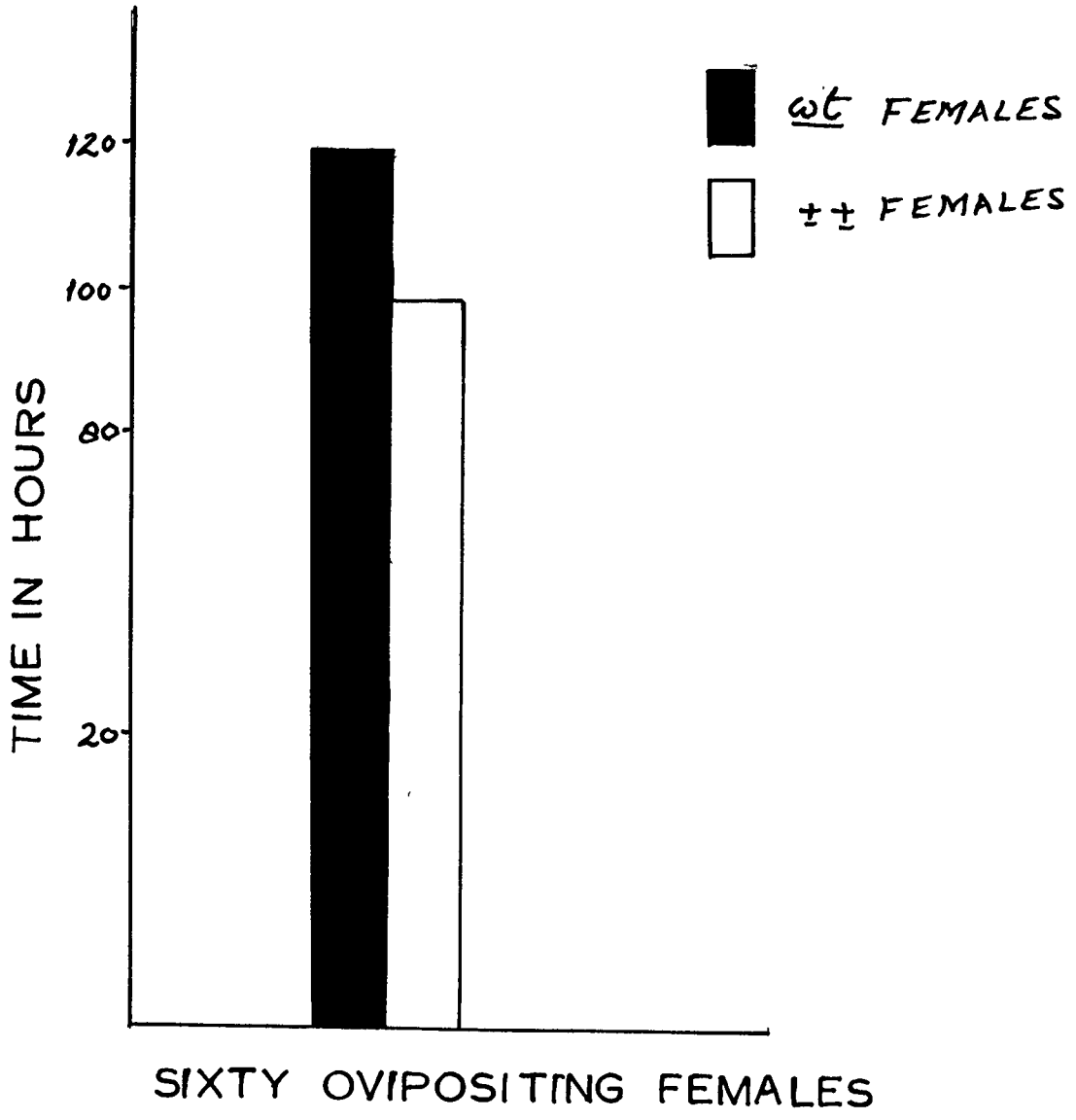
Twenty single pair crosses of each genotype were made. The adults were of uniform age (72 ± 12 hours). First blood feed was provided from anesthetized albino rats on the 4th day of emergence. Subsequent blood meals were given only after the oviposition was completed in all the cages of the two genotypes. As indicated earlier,

Table 9: Time taken for oviposition by the wild type and white thorax genotypes (ROCK strain).

Genotypes	Oviposition time in hours* after blood meal	
	Mean	Range
wild type (+ +)	99.9 ± 0.9	96 - 120
white thorax (wt wt)	120.9 ± 1.26	108 - 132

*Based on 60 single-pair crosses.

Figure 6. Mean oviposition time by $\pm$ $\pm$ and wt wt females (ROCK strain).


wt FEMALES
±± FEMALES
120
100
80
20
TIME IN HOURS
SIXTY OVIPOSITING FEMALES

since the mutant females showed a tendency for late oviposition the second and third blood meals could be given only on the 9th and 24th day of emergence. Sugar pads were available at all times during the experiment.

The data (Table 10), indicate that the females of the white larval thorax mutant were not able to stand the effects of repeated blood meals as favourably as the females of the wild genotype. Out of twenty females of the two genotypes, one of each had died after the first blood meal. The effect of second blood meal was very conspicuous on the mutants where fourteen died upto the 21st day of emergence, five of them without depositing any eggs. As against this only one female of the wild genotype died after the 2nd blood meal. The remaining five females of the mutant were all dead after the 3rd blood meal, by the 33rd day of emergence, three of them without laying any eggs. Here again there was no effect on the wild type females, all of whom survived and oviposited after the 3rd blood feed.

The number of eggs deposited by single females also seemed to be affected by the successive blood meals. Even though the eggs were not counted after each oviposition, visual examination of the egg papers showed a marked reduction in the number of eggs after the 2nd and 3rd blood meals in the wt wt genotype. This effect on fecundity of the wild females was not so marked and they seemed to oviposit almost the same number of eggs after the Ist, 2nd and 3rd blood meals.

Table 10: Effect of successive blood meals on the survival of females as measured by the mortality of adult females after each blood feed.

Blood meals	Days after emergence	Mortality of adult females* ± ±		wt wt	
		after oviposition	with out oviposition	after oviposition	without oviposition
I	4	-	-	-	-
	5	1	-	-	-
	6	-	-	1	-
II	9	-	-	-	-
	10	-	1	4	-
	11	-	-	-	3
	20	-	-	3	2
	21	-	-	2	-
III	24	-	-	-	-
	26	-	-	-	3
	33	-	-	2	-

*Data based on 20 single-pair crosses.

The results of the experiment thus indicate, even though not conclusively, that there is a marked effect of repeated blood meals on the survival and ovipostion of the mutant females. The difference in this regard, between the two genotypes is very evident.

B. Fecundity

The number of eggs laid in a batch by a single female varies greatly between species and several factors may affect the size of the egg batch in any species (Clements, 1963). Egg production by individual females of + + and wt wt genotypes was studied in the ROCK strain. Fifteen single pair crosses were made for each genotype. The adults were of uniform age (72 ± 12 hours). Females were given a single blood meal on an anesthetized rat and glucose pads were available at all times. Egg papers were collected five days after the blood meal. The number of eggs deposited per pair was counted and recorded (Table 11). One or two such crosses where the females did not lay any egg or had died before the egg papers were collected, were discarded and have not been included in the calculations. A normal probability distribution was assumed in determining the mean number of eggs per female.

As would be clear from the data (Table 11), the mean was higher (74.6) for the wild type females than that of the white thorax genotype (66.3). The same is true for the range, 30 - 110 for the wild type while 30 - 90 for the white thorax females. Apparently the data thus indicate a difference in the egg production of the individual females. But, when analysed statistically, the difference in fecundity of the two genotypes is not significant.

Table 11: Fecundity of wild type and white thorax genotypes based on mean number of eggs deposited per female (ROCK strain).

Genotype	Number of eggs deposited*		
	Total	Number per female	
		Mean	Range
wild type (+ +)	1,119	74.6 ± 6.0	30 - 110
white thorax (wt wt)	796	66.3 ± 3.5	30 - 90

*Data based on 15 single-pair crosses of each genotype.

C. Hatchability

In order to determine the difference, if any, in the hatchability of the two genotypes, newly deposited eggs were collected from 15 single pair crosses of each homozygous genotype (+ + and wt wt) from the ROCK strain. To ensure proper embryonation and conditioning, the eggs were stored at controlled conditions ($27^{\circ} \pm 1^{\circ}$ C and 78 ± 8% relative humidity) for four days before hatching. They were then immersed in deoxygenated water and the hatching medium was changed twice after every 24 hours to promote a complete hatch of viable eggs. Unhatched eggs were dissected and those found not embryonated were excluded from the count. The per cent hatch per female was calculated (Table 12).

A difference in per cent hatchability of the wild type and the mutant was indicated. The mean hatch, 68.2%, for the wild type was considerably higher than the mean hatch, 44.1%, for the mutant genotype. The same was true about the range of per cent hatchability of the two genotypes. As would be clear from the data, the difference in hatchability of the eggs of the two genotypes is statistically significant and may, therefore, favour the wild type under these conditions.

Table 12: Hatchability of fresh eggs of wild type and white thorax genotypes based on mean number of larvae hatched per oviposition.

Genotype	Eggs*		
	Total	% hatch per female	
		Mean	Range
+ + (wild)	992	68.2 ± 5.5	26.2 - 87.2
wt wt (white thorax)	802	44.1 ± 4.9	20.2 - 71.8

*Data based on 15 single-pair crosses of each genotype.

D. Larval development

Larval development time and mortality in immature stages were compared for the two genotypes under different conditions of feeding.

1. Development and mortality under optimal conditions:

Time to pupation and mortality during immature stages of the two genotypes (ROCK strain) were determined under optimal conditions of feeding, temperature and humidity. Two pans of newly hatched 100 larvae were set up for each of the + + and wt wt genotypes. They were fed under the usual laboratory schedule. This consisted of one tablet of yeast on day first following hatching, one tablet on the third day, one on the fourth and one tablet on the sixth day. Three replicates of the experiment were made. Time to pupation and mortality in immature stages were recorded. Average per cent mortality and the mean time to pupation were calculated (Table 13).

Even under optimal conditions of feeding and environment, difference, even though slight, between the two genotypes in the time taken for pupation was noticed. The mean time taken for pupation by the + + genotypes was 8.8 ± 0.13 days with a range of 7 - 11 days. For the wt wt genotype it came out to be 9.7 ± 0.1 days and 8 - 12 days respectively. Apparently these differences are slight, but when analysed statistically, they are significant, the wild genotype taking less time and pupating earlier than the white thorax larvae. Similarly, mortality during the immature stages before pupation was higher, 16 per cent, for the mutant larvae than that of the wild type larvae where it was only 11 per cent.

Table 13: Larval development and mortality in immature stages under optimal conditions of feeding and environment (ROCK strain).

Genotype	Days to pupation*		% mortality in immature stages
	Mean	Range	
+ +	8.8 ± 0.13	7 - 11	11
wt wt	9.7 ± 0.1	8 - 12	16

*Based on three replicates, each with 100 larvae.

2. Effect of excess of food on larval mortality and development:

The effect of excess of food on larval mortality and development was studied on the two genotypes of ROCK strain. One pan with 100 larvae in 1,500 ml. of water was set up for each of the two genotypes + + and wt wt. Instead of the usual laboratory schedule of feeding that consisted of one tablet of yeast for 100 larvae on alternate days, these larvae were given four tablets of yeast on the first day, four on the third and again four tablets on the fifth day. The experiment was conducted under optimal conditions of temperature and humidity (27° C ± 1° C and 80% ± 10% relative humidity). Three replicates of the experiment were made.

Parameters determined included time to pupation and mortality in the immature stages (Table 14). Only a few larvae, four and five of the two genotypes + + and wt wt respectively, could pupate, giving a very high rate of mortality in the immature stages. The time taken for pupation was six days in both cases. Similarly, there was no difference in mortality which, being 96% for the + + and 95% for the wt wt, was high in both the genotypes.

Table 14 also illustrates the comparative performance of the two genotypes under conditions of normal and excess feeding. While time to pupation was reduced under excess feeding for both the genotypes, mortality was considerably increased when compared to conditions of normal feeding. This seemed to be due to the excess of scum formation which none of the two genotypes were able to overcome.

Table 14: Effect of excess of food on development and larval mortality under optimal conditions of temperature and humidity (ROCK strain).

Genotypes	Mean pupation time in days**		% larval mortality	
	Normal* feeding	Excess** feeding	Normal* feeding	Excess** feeding
+ +	8.8 ± 0.13	6.0 - 0	11	96
wt wt	9.7 ± 0.1	6.0 ± 0	16	95

*From Table 13

**Based on three replicates, each with 100 larvae.

3. Effect of starvation on development and larval mortality:

One hundred first instar larvae of the two genotypes (+ + and wt wt) of ROCK strain were kept in 1,500 ml. of distilled water without any food to see the effect of starvation on development, larval mortality and survival. Since micro-organisms are the principal constituents of larval diet in most of the species of mosquitoes (Clements, 1963), the distilled water was changed every alternate day in order to avoid the growth of micro-organisms in the medium as far as possible. The larvae were maintained under optimal conditions of temperature and humidity (27° C ± 1° C and 80% ± 10% relative humidity). Mortality was recorded every day and the dead larvae were removed from the pans. Three replicates of the experiment were made.

The mean survival time of the larvae under starved condition was calculated on the basis of the day where 50 per cent of the larvae died in the three replicates. This is represented by LT-50 in Table 15.

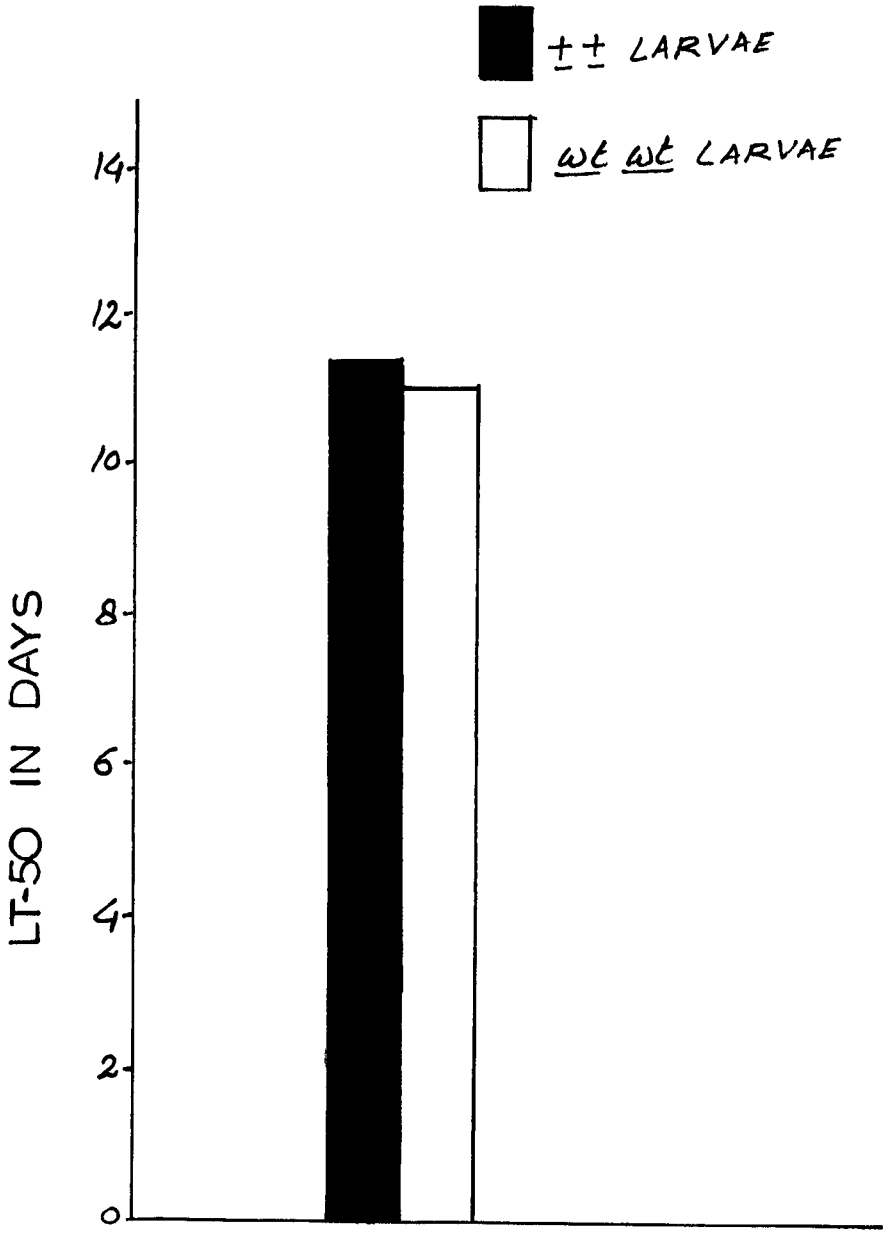
As would be clear from the data presented in Table 15, Figure 7, none of the larvae from the two genotypes were able to pupate under starved conditions. Mortality in immature stages before pupation was 100 per cent in both cases. A slight difference in the range of survival and mean survival time of the larvae as indicated by the LT-50 was noticed between the two genotypes. These differences however, being very small, are statistically not significant.

Table 15: Effect of starvation on survival of larvae of the two genotypes as measured by LT-50 for 100 larvae (ROCK strain) under optimal condition of environment.

Genotype	LT-50 in days*	Range of survival* in days	% mortality before* pupation
+ +	11.6 ± 0.77	0 - 38	100
wt wt	11.2 ± 0.83	0 - 33	100

*Based on three replicates, each with 100 larvae.

Figure 7. Survival of larvae under starvation (ROCK strain).


± ± LARVAE
wt wt LARVAE
LT-50 IN DAYS
14
12
10
8
6
4
2
0

4. Effect of larval density:

It is a known fact that overcrowding affects animals in various ways. An experiment was designed to study the effect of larval density on development and mortality of the two genotypes (+ + and wt wt) in ROCK strain under optimal conditions of environment and feeding. Care was taken to ensure that there was no shortage of food or scum formation in the cultures throughout these experiments. Newly hatched larvae of the two genotypes were divided into lots of 25, 50, 100, 200 and 300. These were placed in small bowls of 5" diameter and 3" depth containing 200 ml. of tap water. The lots having 25 and 50 larvae in the bowl were given one tablet of yeast, those with 100 and 200 larvae two tablets and those with 300 larvae three tablets on the day following hatching. Thereafter the cultures were checked every day and food was added according to the needs of each lot. Dead larvae were removed from the culture every day. Three replicates of the experiment were made. Time to pupation and per cent mortality during the larval stages were compared for the two genotypes.

The results (Table 16) indicate no significant difference in the growth rates of the two genotypes when there were 25 larvae in the bowl. At the density level of 50 and above the differences in the two genotypes were, however, significant in favour of the wild type. At each one of the 50, 100, 200 and 300 density levels the white thorax larvae took longer time to reach pupation than the wild type larvae.

Mortality during the immature stages was similar in the two genotypes when there were 25 and 50 larvae in the bowl. At higher

Table 16: Effect of larval density on development and survival of the two genotypes (ROCK strain).

No. of larvae in bowl	Genotype	Days to pupation* Mean	Range	% mortality in immature stages
25	+ +	6.03 ± 0.08	5 - 7	0.0
	wt wt	6.1 ± 0.07	5 - 8	0.0
50	+ +	5.9 ± 0.06	5 - 7	5.3
	wt wt	6.5 ± 0.06	5 - 8	6.6
100	+ +	6.8 ± 0.04	6 - 8	24.0
	wt wt	7.9 ± 0.05	7 - 9	30.3
200	+ +	8.01 ± 0.21	7 -10	30.6
	wt wt	9.3 ± 0.04	8 -11	33.0
300	+ +	10.3 ± 0.04	8 -14	31.9
	wt wt	11.5 ± 0.05	9 -15	36.6

*Data based on three replicates of each genotype at all densities.

densities, i.e., when there were 100, 200 and 300 larvae in the bowl, mortality was considerably increased in both the genotypes. The differences in mortality were again significantly higher for the white thorax larvae than those of the wild type larvae.

E. Sex ratio:

Sex ratio of adults of + + and wt wt genotypes was studied in ROCK strain. Eggs from 10 single pair matings of each genotype were collected. For proper embryonation and conditioning they were held for four days. They were then hatched and reared under optimal conditions of temperature, humidity and feeding. Sex was examined at the pupal stage and the adults were rechecked after emergence. The results are summarized in Table 17.

Sex ratio in the wild type was close to 1:1 (43.4% females), but it was rather distorted in favour of males in the mutant where the females numbered only 35.3 per cent of the total progeny. The differences in the sex ratio of the two genotypes were significant.

Table 17: Sex ratio in wild type and white thorax genotypes (ROCK strain).

Genotypes	% females in adults*	Range of females
+ +	43.4 ± 1.6	38.8 - 52.4%
wt wt	35.3 ± 1.8	27.5 - 45.1%

*Data based on the mean for single egg batches from 10 single pair matings.

VII. DISCUSSION

The present mutant, white thorax larva, is an addition to the known variants of larval colour in Aedes aegypti. Craig and Gillham (1959) isolated three distinct, genetically controlled colour varieties dependent on the nature and distribution of certain granules in the fat body cells of the larvae. While Wiggelsworth (1942) had described the accumulation of granules of uric acid in the cells of the larval fat body as an excretory mechanism, Craig and Gillham assumed the same granules responsible for the colour variation of the larvae. According to them the predominant class or the wild type which is dark grey to greenish brown, has a uniform distribution of these granules throughout the larval body. The most common variant, a pale yellow form, is devoid of these granules. The third type, melanotic larvae, is a rare variant. These are dark brown to deep black due to an excess of deposition of the granules in the cells of the fat body. White thorax larva is a new variant, quite different from the other three forms. Even though it might be considered very close to the mutant yellow larva, it is distinctly different from it. In the case of yellow the whole body is pale or white while in the present mutant it is only the thoracic region which shows albinism and the rest of the body is more or less exactly like the wild type larvae. The gradual changes in colour with the change in the amount of granules present in the fat body during starvation experiments demonstrated a close relation between the granules and

larval colouration in this case also. The presence of these granules in abundance in the whole body of unstarved wild type larvae and only in the abdominal segments of the mutant while their complete absence in the thoracic segments of the white thorax larvae is a further evidence of the same. Adhami (1964), while working on the nature of yellow larva, confirmed the hypothesis that larval colouration in Aedes aegypti is due to the deposition of uric acid granules in the fat body cells. The same techniques used by him to determine the nature of the granules were followed in this case also. Appearance of deep greenish-blue spots, characteristic of uric acid, in the abdominal segments of the present mutant when stained with carmine and methylene blue, and complete absence of the stain in the fat body cells of the thoracic segments further confirm the uric acid nature of these granules beyond doubt.

Data of breeding experiments on the mechanism of inheritance clearly indicate that white thorax larval character is produced by a single autosomal factor which is recessive to wild type. Crossing experiments between the homozygous wild type and white thorax individuals gave a perfect 3:1 ratio in the F_2 (Table 3) while the testcross progeny of the F_1 heterozygotes yielded the expected 1:1 ratio (Table 4), confirming a monofactorial recessive pattern of inheritance for the white thorax. Reciprocal crosses giving similar results (Table 3 & 4) ruled out the possibility of sex-linkage for this factor. The gene for yellow larva (y) on linkage-group II was employed as a marker in order to determine the linkage group of the

wt gene. Since yellow and white thorax both characters affect the larval colour in a similar manner and since in the presence of y y genes a white thorax larva always looked like a yellow, efforts to obtain a double mutant showing both the characters in the larvae could not succeed. A test-cross with the double homozygous recessive characters, therefore, could not be made. The F_2 progeny of crosses between the yellow and white thorax individuals gave a 2:1:1 phenotypic ratio in all such experiments instead of a typical 3:1 ratio, which could have been expected only if the two genes had been segregating independently. These results are a positive indication of the two genes being linked i.e., the gene wt also being situated on the same linkage group II. A striking observation in this regard was the complete absence of double mutants in the F_2 progeny which should have been formed as a result of crossing over between the two loci. This could be explained only due to the fact that a double mutant does not show the white thorax characteristic in the presence of yellow phenotype. These double mutants must have, therefore, been scored with the yellow phenotypes. This assumption is supported by a close look on Table 5 and Table 6 where one finds that even though the ratio of yellow to white thorax in the F_2 progeny was quite close to 1:1, the number of yellow phenotypes was slightly higher. The increased number of yellow phenotypes could possibly be due to some double mutants having been scored as yellow. The slight difference in the number of yellows to white in the F_2, which is statistically not very significant, could also suggest that the loci

of the two genes y and wt are very close to one another. It would, however, not be proper to arrive at such a conclusion by these experiments and some more markers, other than the y gene,would be required to work out the distance relationship of the wt gene with other genes on chromosome II. Even though crossing over occurs in both sexes with almost similar frequency (Craig and Hickey, 1967), McClelland (1962a), working with certain traits on linkage group I, reported a slightly reduced rate in females. Moreover, variations in linkage distances have also been reported by some workers (Craig and Hickey, 1967). Craig and Hickey have, therefore, suggested that tests on linkage should be made with heterozygous males as far as possible and that data from backcrosses should be preferred over the data from F_2. They have further expressed the desirability of making three-point testcrosses for linkage tests in order to facilitate subsequent mapping. Multiple markers like Wart-S-DS (Wart palp, spot abdomen and dark scutum, Mosquito Genetics Project, Univ. of Notre Dame, Indiana, Strain List of 1969) may prove useful for such studies on the present mutant. Further work on these lines is planned.

The F_1 progeny of a cross between white thorax and yellow was always wild type in all the breeding experiments without exception. It never yielded either one of the two phenotypes or an intermediate between the two. It is, therefore, certain that, although both genes control the deposition of uric acid granules in the fat body cells and therefore, the colour of the larvae, they are not alleles. It is as if the gene y does not allow the accumulation of these granules

in all parts of the larval body whereas the wt gene prohibits its accumulation only in the cells of the thorax.

There is yet another difference between the mechanism of inheritance of the yellow larval colour and that of the white thorax larvae. The dominance of wild type in the case of yellow larvae is not complete in the sense that heterozygotes (y^+y) usually appear lighter than the homozygotes and are apt to form phenocopies of yellow under environmental stress. (Craig and Gillham, 1959). No such phenomenon was noticed in the mechanism of inheritance of the white thorax character. The dominance of wt^+ over wt is complete so that heterozygotes (wt^+ wt) in this case are always wild type indistinguishable from the homozygotes (wt^+ wt^+). Under environmental stress such as over-crowding or improper feeding these heterozygotes do not produce phenocopies of the white thorax. Chemical assay of uric acid content of the various genotypes affecting larval colour may, however, indicate a direct relationship between the type of genes present and the quantity of uric acid produced, deposited and discharged. These mutations could, therefore, be used as tools for physiological studies of gene action.

The data on gene frequency demonstrate that strains vary in the frequency of wt gene and there could be low as well as high frequency populations for this character. Of the four strains where the frequency of this gene was determined in the laboratory populations, ROCK exhibited the highest frequency of 0.65 while in KUALA it was the lowest (0.20). NIH and NEW MICK came in between the two with a

frequency of 0.39 and 0.32 respectively. These frequencies of the wt gene were compared with the frequency of y gene in each one of the four strains. It is strange to note that the strain which showed the highest frequency of wt gene, (i.e. ROCK) had also the highest frequency (0.50) of the y gene and the strain KUALA which had the lowest frequency of wt gene, had the lowest frequency (0.27) of the y gene as well. Again NIH and KUALA exhibited gene frequencies for y in between the two, 0.38 and 0.37 respectively. The frequencies of the wt gene even though different in different strains are such that the gene can be deemed to be of common rather than of rare occurrence in populations of Aedes aegypti. Adhami (1964) indicated a high rate of mutation from y^+ to y and none or almost negligible reverse mutation from y to y^+ in the case of yellow larva. The same could be the case here. Even though the rate of mutation from wt^+ to wt was not studied, a rough estimation of back mutation (Table 8) did indicate that it was, if at all, of very rare occurrence since no reverse mutation from wt to wt^+ could be detected among 43,264 gametes tested. A high mutation rate from wt^+ to wt and very low rate of back mutation would satisfactorily account for the common occurrence of the white thorax larvae in populations of Aedes aegypti. It is planned to take up the study on mutation rates later.

Since the frequency of mutation at a particular locus may be affected by various hereditary factors on the genome and different strains have different genetic backgrounds, the differences in the

frequency of mutation and, therefore, in the frequency of wt gene in different strains, are not altogether unexpected. Demerec (1941) and Dobzhansky et al.(1952) have reported similar phenomenon in different populations of Drosophila.

It would be interesting to follow the frequency of the wt gene both between strains and within a strain over a period of time and to see the effect of selective pressures on different populations. Population studies of Adhami (1964) with the yellow larva confirmed the earlier suggestions (Craig and Gillham, 1959, Craig, Hickey and VandeHey, 1961) that the gene y showed different levels of balanced polymorphism in different strains and at different laboratories. It would be worthwhile to determine if the same is the case with the wt gene. Is there any equilibrium point or plateau for the frequency of this gene in experimental populations and, if so, do the strains vary in their equilibrium points as they do in the frequency of the gene? Further work on these lines, which promises a close insight into the behaviour of the gene in populations, is planned.

While preliminary studies of Craig and Gillham (1959) and Adhami (1962) had indicated that the gene causing yellow larva was disadvantageous under some conditions, Adhami (1964) showed that the yellow phenotype had an adaptive advantage atleast under certain conditions.

Factors which might give one of the genotypes a competitive advantage, in the case of white thorax larval mutant and the wild type, were sought. The competitive ability of the two genotypes was

compared under different conditions and at different stages of the life cycle. The results are summarised in Table 18.

Experiments on the behaviour of oviposition by the females of the two genotypes indicated that the wild type ($\underline{wt}^+$ $\underline{wt}^+$) females were able to lay full complement of eggs in a relatively shorter time after the blood meal than the females of the mutant ($\underline{wt}$ $\underline{wt}$) (Table 9). The females of the mutant seemed to hold back the eggs for a little while before ovipositing. It is a known fact that oviposition in mosquitoes may be delayed or prevented by cold (Nicholson, 1921; Mayne, 1926a), absence of water (Fielding, 1919; Holstein, 1952; Woke, 1955) or by failure to mate (Macfie, 1915; Mer, 1936; Tate and Vincent, 1936). Since the experiments in the present case were conducted in an environmental control room and under optimal conditions, the environmental factors influencing oviposition may be ruled out. It would, however, be interesting to compare the mating ability of the adults of the two genotypes and find out if there were any differences in the behaviour of oviposition. Gillet (1955c, 1956a) while investigating the effect of mating on oviposition in two strains, NEWALA and LAGOS, of Aedes aegypti, found that the differences of oviposition behaviour (early or delayed oviposition) had a genetic basis. If this is so it would be worthwhile to see how the females heterozygous for the white thorax larval character ($\underline{wt}^+$ $\underline{wt}$) behave in this regard and is there any corelation between the genetic factor for oviposition and the gene responsible for the white thorax larval trait.

Table 18: Summary of results comparing adaptive advantage of the $\underline{wt}^+$ $\underline{wt}^+$ and $\underline{wt}$ $\underline{wt}$ genotypes.

Characters	Conditions	Genotype at an advantage
Oviposition	Time taken after blood meal, optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$
Survival of females	Effect of repeated blood meals, optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$
Fecundity	Based on single-pair crosses, optimal conditions.	None
Hatchability	Fresh eggs, optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$
Larval development		
Time to pupation	Optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$
	Excess of food	None
	Starvation	None
	Larval density (Crowding)	$\underline{wt}^+$ $\underline{wt}^+$
Larval mortality	Optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$
	Excess of food	None
	Starvation	None
	Larval density (Crowding)	$\underline{wt}^+$ $\underline{wt}^+$
Sex ratio	Adults-larvae reared under optimal conditions.	$\underline{wt}^+$ $\underline{wt}^+$

Mosquitoes usually survive enough to lay a number of batches of eggs. Mathis (1935) showed that females of Aedes aegypti could survive long enough to lay as many as 22 batches of eggs. There could be a large number of factors affecting oviposition behaviour, fecundity and survival of the adult females. Studies on the effect of repeated blood meals on survival and oviposition of the females of the two genotypes revealed that the wild type (wt^+ wt^+) females were able to stand the effects of successive blood meals in a much better way than the females of the mutant (wt wt). The data (Table 10) based on 20 single-pair crosses under optimal conditions showed that none of the 20 females of the mutant could survive after the third blood meal quite a few of them dying without ovipositing. This is in conformity with the observations of Clements (1963) that mortality in the females of Aedes aegypti is increased with repeated gonotrophic cycles. On the wild type genotype, however, the effect of successive blood meals was not so pronounced as 18 of the 20 females survived and oviposited after the third blood meal. The wild type females, therefore, showed to possess a definite advantage over the mutant females in this regard. Another important difference between the two genotypes, noticed by visual examination of the single egg batches, was the marked decrease in the number of eggs laid by the mutant females after successive blood meals. Putnam and Shanon (1934) estimated a decrease of 15% in the number of eggs after each oviposition in Aedes aegypti. No such marked decrease in the number of eggs was noticed in case of the wild type females upto three ovipositions.

The wild type females seemed to have an advantage over their counterpart mutants in this regard also. The performance of heterozygotes ($\underline{wt}^{+}$ $\underline{wt}$) in these respects, which could not be seen due to lack of time, remains to be determined.

The two phenotypes were generally similar in fecundity under optimal conditions (Table 11). There was no significant difference in the number of eggs laid by the females of the two genotypes under normal laboratory conditions. Adhami (1964) showed that the females of the mutant yellow larva were more fecund and produced more eggs than the wild type females under conditions of crowding. He further observed that performance of the heterozygotes was better than either homozygote in this regard. It would be interesting to find out if the gene $\underline{wt}$ also bestows such an advantage or disadvantage to its possessors under conditions of crowding or other abnormal situations. Many workers have pointed out that the size of the egg batch or fecundity in insects is affected by a large number of factors including the amount and type of blood ingested, the size of ovipositing female, the number of ovarioles in the two ovaries and the feeding and environmental conditions of the larvae under which the ovipositing females had been reared. The differential effect, if any, of these factors on fecundity of the two genotypes remains to be determined.

A significant difference, in favour of the wild type, was noticed in the hatchability of the two genotypes. Eggs from the wild type female had a much greater hatchability (68.2%) than those

of the mutant where the mean hatchability was reduced to 44.1 per cent. This is in sharp contrast to the results reported by Adhami (1964) for the yellow larval mutant. According to his observations there is no significant difference in hatchability of fresh eggs between the mutant yellow larva and its wild genotype. He reported a significantly greater survival and hatchability of aged eggs stored at subptimal or varying conditions of temperature and humidity in the case of yellow mutant. It would be interesting to compare the survival and hatchability of aged eggs of the present mutant and its wild type genotype by storing the eggs over a period of time under varying conditions of environment.

There could be a number of factors affecting hatchability. It is a known fact that members of the genus Aedes can resist long periods of drought. This is perhaps due to the structure and function of the coverings of the egg. According to Harwood (1958) and Harwood and Horsfall (1959), the exochorion imbibes water from the saturated environment and releases it slowly while the egg is drying. The chorion, in association with the endochorion, creates a barrier to the movement of water thus keeping the embryo safe from dessication. Kliewer (1961) corelated the decrease in hatchability of the eggs of Aedes aegypti to loss of moisture when the eggs were stored in dry conditions. In the present case, however, since the hatchability of fresh eggs, which had been conditioned under optimal humidity and temperature, was considered, dessication of embryos due to lack of moisture can be ruled out. Exochorion, the outermost covering of the

egg, is a delicate membrane and may be damaged due to internal pressure of the egg or due to some external reasons or injury at the time of oviposition. If the eggs are not properly conditioned and a damage is done to the exochorion, the embryos may not survive in their egg shells even for short periods and this may well account for the decrease in hatchability. In the present experiments, however, all possible care was taken and none of the unhatched eggs revealed any sort of damage to the covering when examined under the microscope.

Craig and Horsfall (1958) found diagnostic differences in size, shape, colour and details of chorionic sculpture of various species of aedine mosquitoes. If the exochorion, which varies in thickness, becomes too thick, it may close the air-channels to the embryo. Thus a short supply of oxygen may not allow the embryo to survive within the egg shell. It would be worthwhile to determine if there are differences in the structure or chorionic sculpturing of the eggs of the two phenotypes studied here. This may provide some clue to the differential hatchability of the eggs of the two genotypes.

The time required for larval development and mortality in immature stages were determined for the two genotypes under different conditions.

Under optimal conditions of environment and feeding a significant difference was noticed between the two genotypes in their development time. The mutant (wt wt) larvae which took 9.7 days on the average to reach pupation showed a slower rate of growth

than the wild type larvae which pupated in 8.8 days on the average. Similarly, mortality during immature stages before pupation was higher (16 per cent) for the mutant larvae than that of the wild type where it was only 11 per cent at optimal conditions of feeding and environment. In this respect again the present mutant differs from the mutant yellow larva which compared well with its wild type under optimal rearing conditions (Adhami, 1964). The performance of heterozygous ($\underline{wt}^+$ $\underline{wt}$) individuals could not be checked in this regard. From the standpoint of population dynamics it would be rewarding to determine whether the dominance of the wild type is complete over its $\underline{wt}$ allele in this selective advantage or it is a case of over-dominance where the heterozygotes do better than either of the homozygotes.

Under conditions of excess feeding the performance of both the genotypes ($\underline{wt}^+$ $\underline{wt}^+$ and $\underline{wt}$ $\underline{wt}$) was more or less similar and there was no significant difference between the two. While time to pupation was reduced for both the genotypes mortality was markedly increased. The medium usually became fouled with scum formation when the food was in excess. It seems none of the two genotypes were able to eliminate or overcome the ill effects of scum formation and most of them died in the fouled medium.

Under starved conditions when no food was available to the developing larvae, no significant differences in the growth rate and mortality of the two genotypes were noticed. The growth rate, as would be expected, was extremely slow and none of the larvae from

either genotype could reach pupation. Mortality during the immature stages was 100 per cent in both cases. The survival time of the larvae as calculated by LT-50 in days (Table 15) was very similar for the two genotypes. The slight difference in the range of survival, where the last of the wild type larvae survived upto 38 days while the mutants lasted only upto 33 days of starvation, was not significant.

Extended development under complete starvation in the larvae of mosquitoes has been noticed by Weilding (1928) and Cristophers (1960). Christophers maintained three separate cultures of early second instar, early third instar and early fourth instar larvae under complete starvation and observed that the increase in the duration of instars was directly related to the number of days of starvation. It seems logical to assume that the second, third and fourth instar larvae obtained enough nourishment and had enough reserve material to sustain them till pupation. Subsequent starvation only extended their development proportionally to the days of starvation. In the present case since the larvae were starved from the very beginning, ever since they hatched, it is not surprising to note that none of them could survive long enough to reach pupation.

The two genotypes were tested for their response to crowding during larval stages. The white thorax phenotype compared well with the wild type when there were only 25 larvae in the bowls. Development at higher density levels was slow in both the genotypes. However, under crowded conditions with no shortage of food the wild type larvae grew comparatively faster than the white thorax larvae. At the

density levels of 50 and above the difference in the growth rate of the two genotypes was significant, the wild type having an edge over the mutants. Similarly mortality during the immature stages at density levels of 100 and above was comparatively higher for the mutant than for the wild type larvae (Table 16). The results indicate that the white thorax phenotype is at a disadvantage under conditions of crowding during immature stages. Similar results have been reported by Adhami (1964) for the mutant yellow larva when he compared them with their wild type under conditions of crowding.

Since the larvae may obtain their nourishment from the microorganisms even when no food is available in the medium, the differential growth of microorganisms makes results from replicate cultures rather variable. Some workers have suggested the use of sterile cultures when standardised or uniform individuals are required for physiological or toxicological studies. Aseptic conditions have been employed by Trager (1935a, 1935b) and Akov (1962) in their studies on larval nutrition and the effects of larval density. It would be interesting to repeat the present experiments on starvation and larval density under sterile conditions and to determine if there were any differences in the nutritional requirements of the two phenotypes.

Differences in the sex ratio of adults of the two phenotypes were found in the ROCK strain. In the wild type sex ratio was close to normal (1:1) but it seemed to be slightly distorted in favour of males in the mutant. In this way again the white thorax larval mutant

showed a difference from the mutant yellow larva. Adhami (1964) reported sex ratio close to 1:1 for the yellow and its wild type phenotype in the ROCK strain. It is, however, interesting to note that just reverse was the case in TEXAS strain where he found a marked difference between the two genotypes in this regard. He observed an almost normal (1:1) sex ratio in the yellow mutants but a much distorted ratio in favour of males in the wild genotype of this strain.

The skewed sex ratio in the present mutant could be due to the presence of a male-producing factor (distorter) described by Craig, Hickey and VandeHey (1960), Hickey and Craig (1962), Hickey (1964) and Hickey and Craig (1966). It could be that this factor became accidentally fixed in the mutant stock during the course of inbreeding to isolate and establish a pure line. However, as the male-producing factor and the gene for white thorax larva seem to lie on different linkage groups, the male-producing factor being on Chromosome I (Hickey and Craig, 1966), it seems rather improbable that this would affect the success of larval phenotypes. It would be rewarding to study and compare the sex ratio between the two genotypes in other strains of Aedes aegypti where white thorax larval mutant has been found. This might throw some light for a better understanding of the correlation, if any, between the two different genes.

The present studies demonstrate that the new larval mutant, white thorax, is at a comparative disadvantage over its wild phenotype

under most of the laboratory conditions. However, for a better understanding of the gene causing white thorax larva a more detailed study of some of the apparently trivial characters will have to be made. Performance of the heterozygotes in different stages of the life cycle and under different conditions of feeding and environmental stress should be determined and compared with either homozygote. If the heterozygotes acquire an advantage over either homozygote, the present mutant could also show genetic polymorphism in different populations of Aedes aegypti as has been reported for the mutant yellow larva (Adhami, 1964). The behavioural differences between the genotypes should be studied. Schoenig (1967, 1968, 1969) while studying the behaviour of movement in two strains of Aedes aegypti found that the yellow phenotypes were slower than the wild type adults. If similar is the case with the present mutant this may cause a difference in the dispersal and other activities of the two phenotypes. Longevity of adults, mating ability of the different genotypes and an estimation of mutation rates from wt^+ to wt and vice versa are some of the important studies that should be made. The results of these studies may provide an explanation, at least in part, for the abundance or scarcity of one of the two phenotypes in populations. A detailed information of the frequencies and behaviour of the gene in populations is thus expected to provide a better understanding of the bionomics of the species. Further work along these lines has been planned for the future.

VIII. CONCLUSIONS

1. A new larval mutant, white thorax, has been found, isolated and established in different strains of Aedes aegypti. It is different from yellow, brown and melanotic larval mutants described earlier. The normal or wild type colour of the larvae is dark greyish and is caused by the deposition of uric acid granules in the cells of the fat body. Yellow larva is due to complete absence of these granules while the albinism of white thorax results by the absence of uric acid granules from the thoracic region only.

2. Like the yellow larval mutant, the white thorax larva is also controlled by an autosomal recessive gene situated on linkage group II. The two genes, however, have different loci and are nonallelic.

3. Different strains of Aedes aegypti show different gene frequencies for the white thorax larva, ranging from 0.2 to 0.65.

4. The variation in larval colour is due to a mutation from wt^+ to wt. Apparently there is little or no mutation in the reverse direction.

5. Selective forces favouring the two phenotypes were sought. The wild type has an adaptive advantage under stress and most of the laboratory conditions.

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